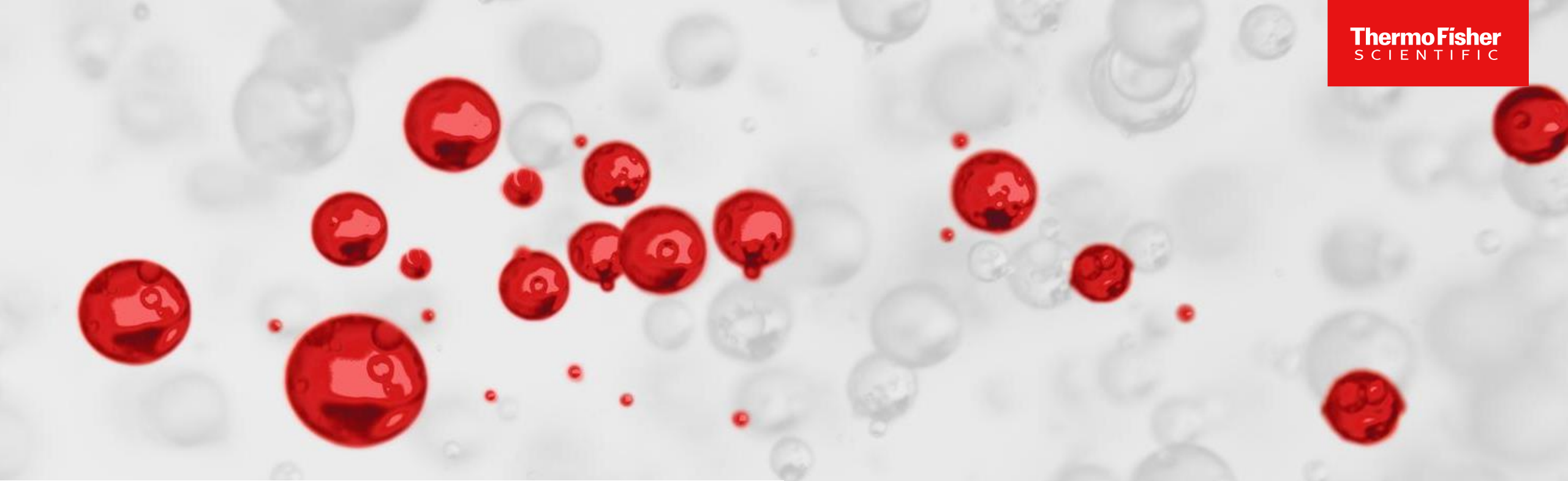
Infectious pathogen detection from wastewater samples

The world leader in serving science

Agenda

1. Introduction to wastewater surveillance and our solutions
2. Detection of SARS-CoV-2 in wastewater samples from collection to analysis
3. Workflows from Thermo Fisher Scientific for SARS-CoV-2 detection from wastewater samples



Introduction to wastewater surveillance and our solutions

What is wastewater and sludge?

Wastewater

also referred to as sewage, includes water from household and commercial building use (e.g., toilets, showers, sinks) that can contain human fecal waste, as well as water from non-household and non-commercial building sources (e.g., rainwater and industrial use)

Sludge

is the residual, semi-solid material that is produced as a by-product during treatment of wastewater

What is wastewater and sludge?

Certain pathogens are shed in fecal waste (stool), which eventually aggregates with other particulates in pooled wastewater and becomes what we refer to as sewage

Wastewater can be tested for genetic material from pathogens that cause infectious diseases and sickness

Wastewater testing has been successfully used as a method for early detection of diseases other than that caused by SARS-CoV-2, such as polio

Purpose of wastewater surveillance

Testing wastewater allows detection of the pathogen before outbreak of the disease occurs

Captures a broad and diverse sampling of stool from municipal wastewater treatment plants; facilities such as aged care homes, student dorms, quarantine facilities; and passenger groups on international flights and cruise ships

Allows administrations to respond before an outbreak spreads further

Depending on the frequency of testing, wastewater surveillance can be a leading indicator of changes in infection in a community

Wastewater surveillance is now being used for SARS-CoV-2

Examples of wastewater testing for SARS-CoV-2

Transmission of SARS-CoV-2 in the Wider Environment Group (TWEG)

Evidence of wider environmental transmission of SARS-CoV-2 (June 12, 2020)

- Preliminary study with 12 collaborators across the UK
- £1 million of funding given to Newcastle University, partnered with Northumbrian Water
- Collaboration from an earlier project with the University of Santiago de Compostela, Spain

Wales Environmental Wastewater Analysis and Surveillance for Health (WEWASH) project

Project extension to continue monitoring levels of COVID-19 in wastewater across Wales (link)

A microbiome research lab in San Diego, California

Information on the application of wastewater testing for the detection of SARS-CoV-2

Q&A: Wastewater monitoring with Professor Rob Knight (ucsd.edu)

High-throughput wastewater SARS-CoV-2 detection enables forecasting of community infection dynamics in San Diego county (medRxiv)

COVID-19 daily dashboard (ucsd.edu)

UC San Diego detects coronavirus in wastewater samples from 5 areas of campus (La Jolla Light)

Automated high-throughput viral concentration from wastewater using the Thermo Scientific™ KingFisher™ Flex platform (protocols.io)

Finding worth in waste: How wastewater monitoring helps reduce the spread of SARS-CoV-2 at UC San Diego (ucsd.edu)

Key features

Applied Biosystems™ MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit

with Virus Enrichment

The MagMAX Wastewater Ultra Nucleic Acid Isolation Kit is an automation-compatible extraction kit optimized to extract viral nucleic acids from wastewater, sewage, and sludge. Versions of the kit are available with and without Applied Biosystems™ Dynabeads™ Wastewater Virus Enrichment beads to concentrate your wastewater sample.

Flexibility

1. Choose your preferred concentration method and protocol
2. Pre-concentrate samples from large volumes using ultracentrifugation, precipitation, or filtration
3. Use the Dynabeads option for automated viral enrichment of up to a 10 mL volume
4. Directly isolate from 1 mL of wastewater without concentration
5. Manual and automated protocols available

Whole workflow solution

Purchase the whole workflow from sample collection to analysis with one supplier. We can help you get started on a wastewater testing program.

Scalability

- These automation compatible kits can help streamline your process to set up sampling across the scale of your organization.
- Automation-ready protocols written for KingFisher Flex, Thermo Scientific™ KingFisher™ APEX, and Thermo Scientific™ KingFisher™ Duo Prime systems

Dynabeads

An option of purchasing the kit with Dynabeads Wastewater Virus Enrichment beads so that your concentration process is streamlined and automated as well.

Technical features of MagMAX Wastewater Ultra Nucleic Acid Isolation Kit

Key features

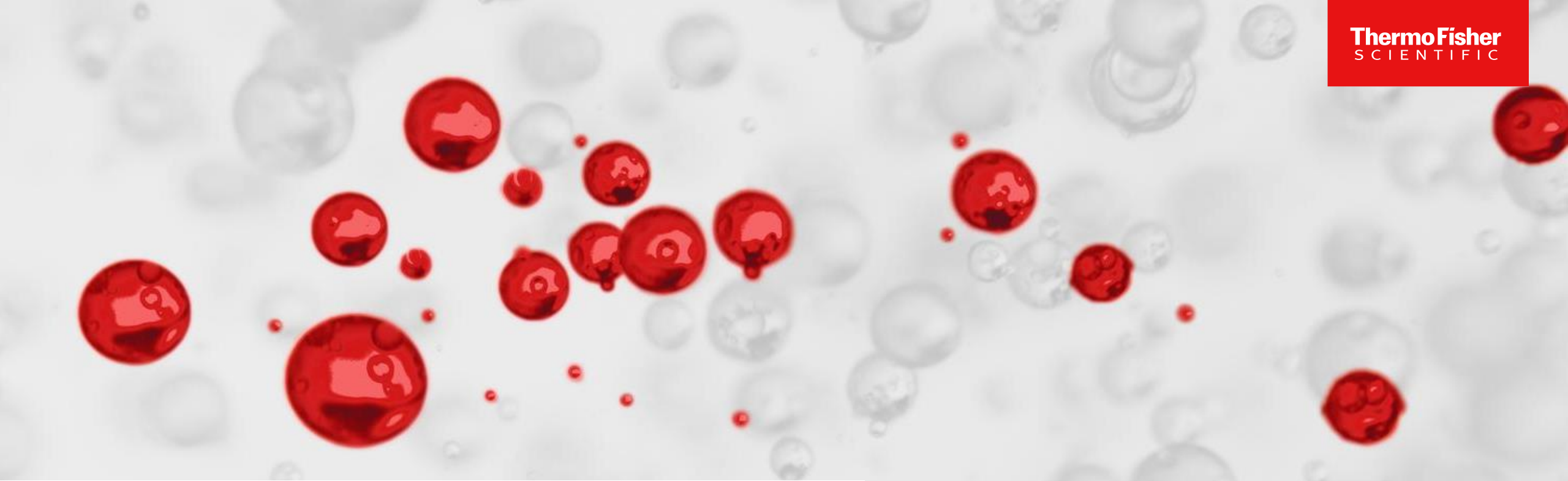
- Flexible protocols enable different up-front concentration methods and input volumes
- Optional virus enrichment workflow
- Full workflow from collection to analysis is available

Sample types	Wastewater and sludge
Concentration and virus enrichment methods	• Compatible with precipitation, ultracentrifugation, and filtration • Virus enrichment of 10 mL wastewater
Sample input volume	• 200 µL – 50 mL (or more) depending on concentration method • 200 mg of sludge
Compatibility	Real-time (quantitative) PCR (qPCR), digital PCR, and next-generation sequencing (NGS) automation-ready protocols designed for KingFisher Flex, APEX, and Duo Prime systems, plus manual protocols
Total processing time	• 45 minutes for nucleic acid isolation including hands-on time • Less than 2 hours for virus enrichment + nucleic acid isolation
Format	20–100 preps depending on input volume
Price per prep	Depends on input volume
Cat. No.	• MagMAX Wastewater Ultra Nucleic Acid Isolation Kit (A52606) • MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment (A52610)

Protocols included in kit

	Wastewater starting volume	Sample details	Kit reagents sufficient for	KingFisher scripts available	Protocol time	Purchase this kit	
1	10 mL	Concentration of 10 mL wastewater using Dynabeads followed by nucleic acid isolation	100 preps	Flex, Duo Prime, APEX	90 minutes	MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment	A52610
2	200 µL	200 µL wastewater pre-concentrated using a preferred method, e.g., ultracentrifugation	100 preps	Flex, Duo Prime, APEX	45 minutes	MagMAX Wastewater Ultra Nucleic Acid Isolation Kit	A52606
3	1 mL*	A. 1 mL non-concentrated wastewater B. Wastewater pre-concentrated to 1 mL volume using a preferred method, e.g., precipitation	20 preps	Flex, Duo Prime, APEX	45 minutes		
4	50–500 mL	Concentration of 50–500 mL wastewater using filtration followed by nucleic acid isolation	20 preps	Flex, Duo Prime, APEX	90 minutes		
5	Sludge	200 mg sludge	20 preps	Flex, Duo Prime, APEX	45 minutes		

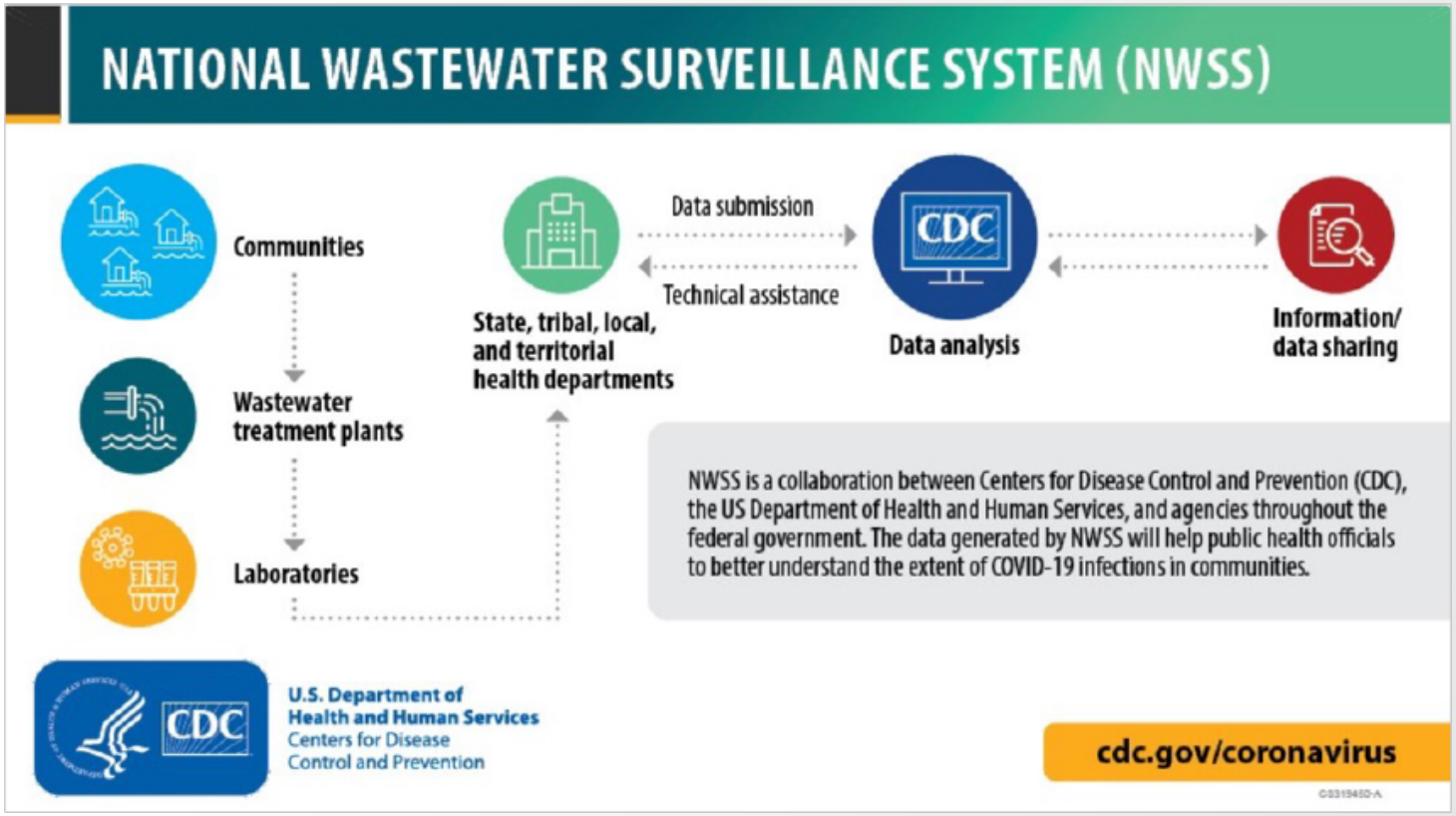
*works for 1-2 mL, but 1 mL is the most sensitive



Detection of SARS-CoV-2 in wastewater samples from collection to analysis

Using wastewater as a sample source

1. Testing wastewater allows detection of the pathogen before outbreak of the disease occurs
2. Wastewater systems transport to wastewater treatment plants
3. Samples of untreated wastewater or primary sludge are collected and sent to environmental laboratories
4. Data are submitted to participating health departments
5. Health departments submit these data to CDC
6. CDC analyze the data in real time and report results to the health department for use in their response



cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance/data-reporting-analytics.html

Wastewater surveillance experimental workflow

Sample collection

Sample processing

Real-time PCR

Analyze and report

Sample types

- Wastewater
- Sludge

Sample processing includes

- Concentration
- Viral nucleic acid extraction

Sample collection

What are the sampling trends and best practices for SARS-CoV-2?

How often should I collect wastewater?

3x/week is commonly used

- Diminishing returns on daily
- Weekly is too long between datapoints

What sampling method should I use?

Grab sampling

- Take a sample at just one timepoint during the day

Composite sampling

- Take samples at multiple timepoints during the day and combine into one bottle
- **Time may vary**
  University dorm–morning flush (3–5 hr)
  Industry–occupied hours

Composite sampling is preferred because it increases the likelihood of detecting virus. If you collect at just one timepoint and no one used the restroom during this time, you may not see anything.

How do I collect the water samples?

Hach™ AS950 Portable Compact Sampler sold through Fisher Scientific™ can be used for easy composite sampling.

Program your days and times

- Contact your Fisher Scientific rep for everything you need to get started collecting wastewater

Concentration

Wastewater itself is a very diluted starting sample. When processing wastewater samples, it is important to use optimal volumes to account for low abundance of viral particles. This often requires a **concentration step** prior to viral nucleic acid extraction.

Recommended technique for wastewater concentration:

Magnetic bead technology*

and filtration

* This method provides the highest sensitivity for viral concentration; see appendix for other concentration methods that perform suboptimally.

Concentration

Virus concentration using Dynabeads magnetic bead technology

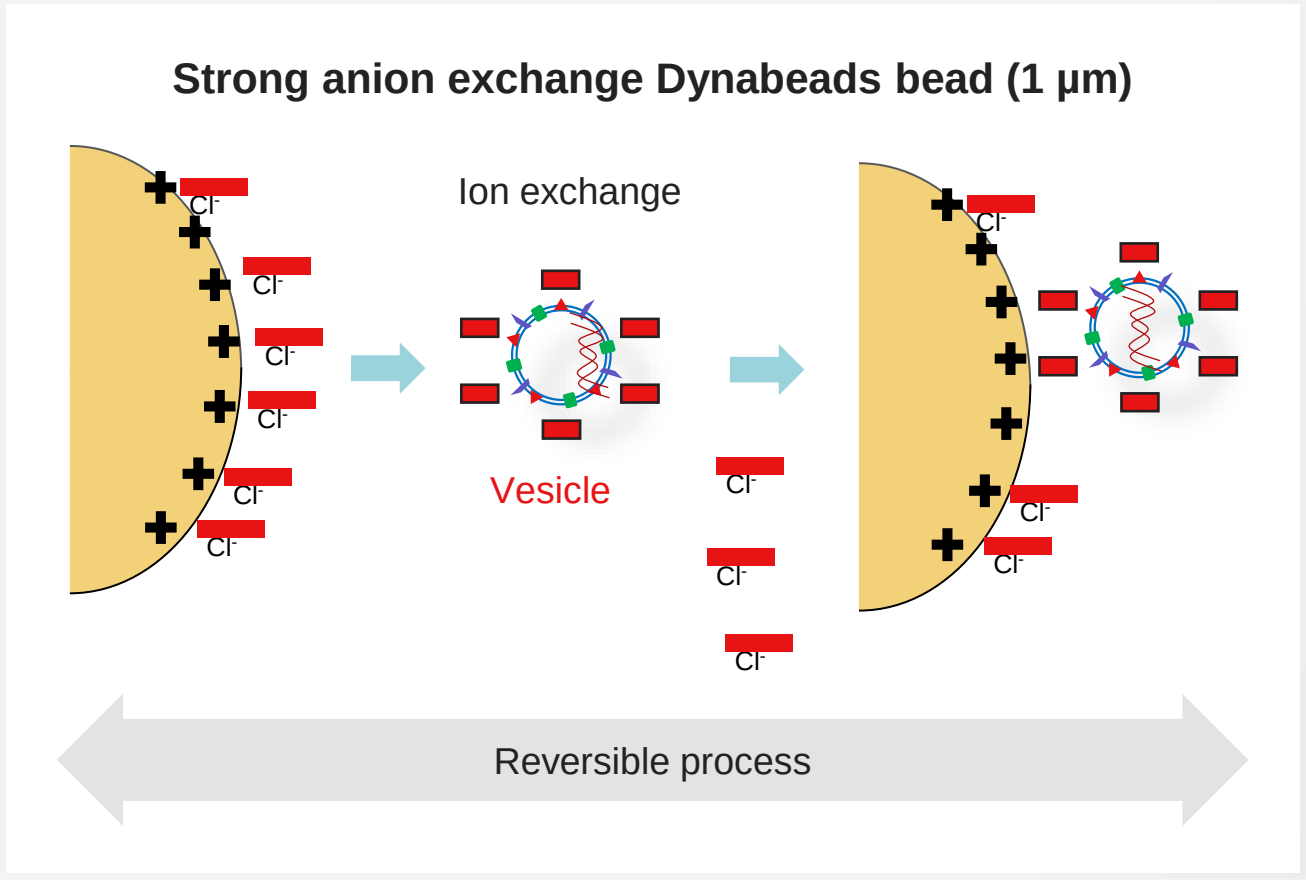
Dynabeads Wastewater Virus Enrichment

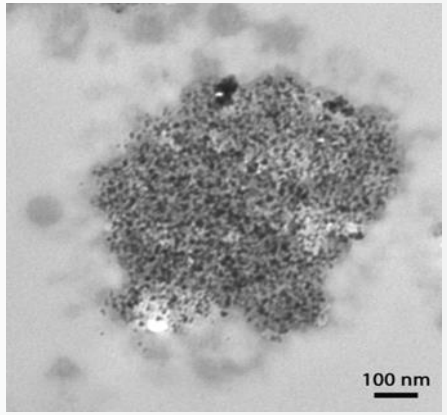
Enables the concentration of intact virus and fragmented RNA in wastewater prior to nucleic acid isolation

Dynabeads Wastewater Virus Enrichment beads are included in the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment, Cat. No. A52610

The isolation principle behind Dynabeads Wastewater Virus Enrichment beads

Magnetic bead–based isolation by charge





- Fast isolation kinetics (~10 min)
- Easy release (~10 min)
- Easy to automate (KingFisher instruments)
- Can be used on other negatively charged enveloped viruses, vesicles, and proteins

Filtration for wastewater virus enrichment

Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Single Use Vacuum Filter Units

Catalog number	Unit size	Material (membrane)
150-0045 and 154-0045	Case of 12	Nylon

Volume (metric) upper	Pore size	Volume (metric) receiver
150 mL and 500 mL	0.45 µm	150 mL and 1,000 mL

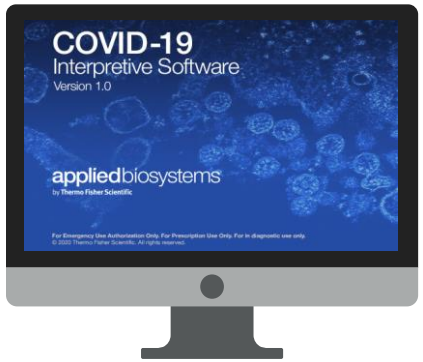
Use Cat. No. 154-0045 1000 mL volume for up to 500 mL

Pathogen detection using PCR

Range of PCR platforms:

1. All Applied Biosystems™ 7500 systems (7500, 7500 Fast, 7500 Fast Dx)
2. Applied Biosystems™ QuantStudio™ 5 instruments (96-well, 0.1 and 0.2 mL block; 384-well block)
3. Applied Biosystems™ QuantStudio™ 7 Flex instruments (384-well block)
4. Digital PCR

Analyze and report



Interpretive software

Applied Biosystems™

Design and Analysis 2.6 software

Recommended products for each step of the workflow

Sample collection

Sample types:

- Wastewater
- Sludge

Sample processing

Automated or manual extraction using the KingFisher Flex, Apex, and Duo Prime Purification Systems

MagMAX Wastewater Ultra kit

PCR

Range of platforms:

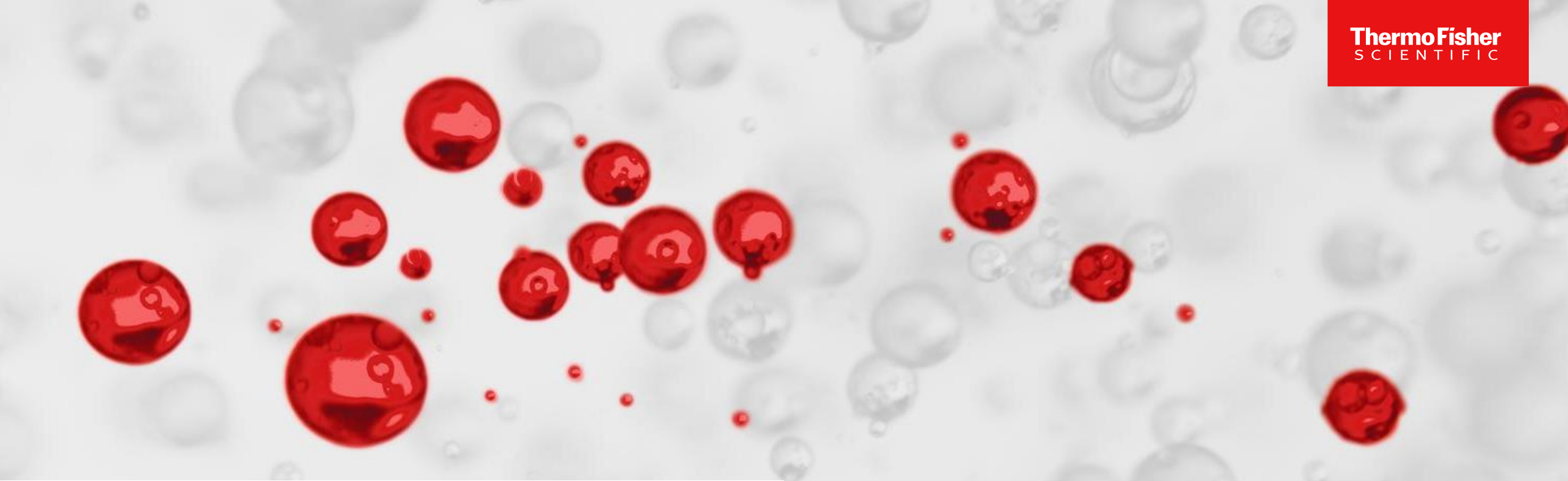
- All 7500 systems (7500, 7500 Fast, 7500 Fast Dx)
- QuantStudio 5 instruments (96-well, 0.1, 0.2 mL block; 384-well block)
- QuantStudio 7 Flex instrument (384-well block)
- Digital PCR
- QuantStudio 12K Flex Real-Time PCR System

Analyze and report

Interpretive software

- Design and Analysis 2.6 software

Intended use of the products in this workflow graphic vary. Please refer to the instructions for use for applicable intended use.



Workflows from Thermo Fisher Scientific for SARS-CoV-2 detection from wastewater samples

Nucleic acid extraction

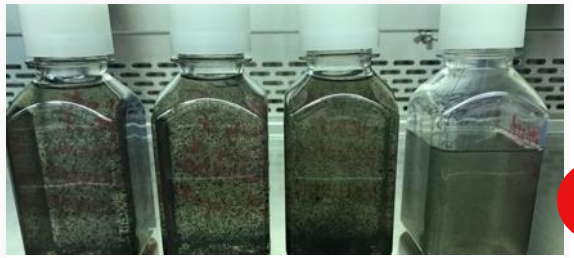
Protocols available with the kit

Different volumes of wastewater require different processing approaches:

1. 10 mL samples with Dynabeads Wastewater Virus Enrichment method for concentration
2. 200 µL samples pre-concentrated via preferred method, e.g., ultracentrifugation
3. 1 mL samples pre-concentrated via preferred method, e.g., ultracentrifugation or precipitation, or direct purification of non-concentrated sample, or water filters
4. >50 mL samples using filtration methods for concentration
5. Sludge (200 mg)

Nucleic acid extraction

1 Automated protocol for 10 mL wastewater samples using Dynabeads for viral enrichment



Wastewater samples

Enrichment of viral material in wastewater samples

Dyna_Flex24_WastewaterEnrich_V2

Note: Pre-processed 10 mL of wastewater with 5 mL wastewater/plate aliquoted in two 24-deep-well plates

and beads added into only one plate (sequential binding and eluted in single plate)

Transfer of the eluate to a 96-deep-well plate

Addition of Proteinase K, binding buffer, and beads from MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment

Extraction of nucleic acid

Downstream analysis

This protocol is the same as the UCSD Knight Lab protocol using Dynabeads beads.

Manual workflow for 10 mL wastewater samples

1 10 mL wastewater samples processed using Dynabeads for viral enrichment

Wastewater samples

Wastewater samples with spike-in of SARS-CoV-2 along with Dynabeads

Binding of viral material to Dynabeads with pre-processed wastewater for virus enrichment; 10 min @ RT

Separation of beads using Invitrogen™ DynaMag™ magnet

Resuspension in lysis buffer

Downstream analysis

Extraction of nucleic acid

Addition of the supernatant to a 96-deep-well plate

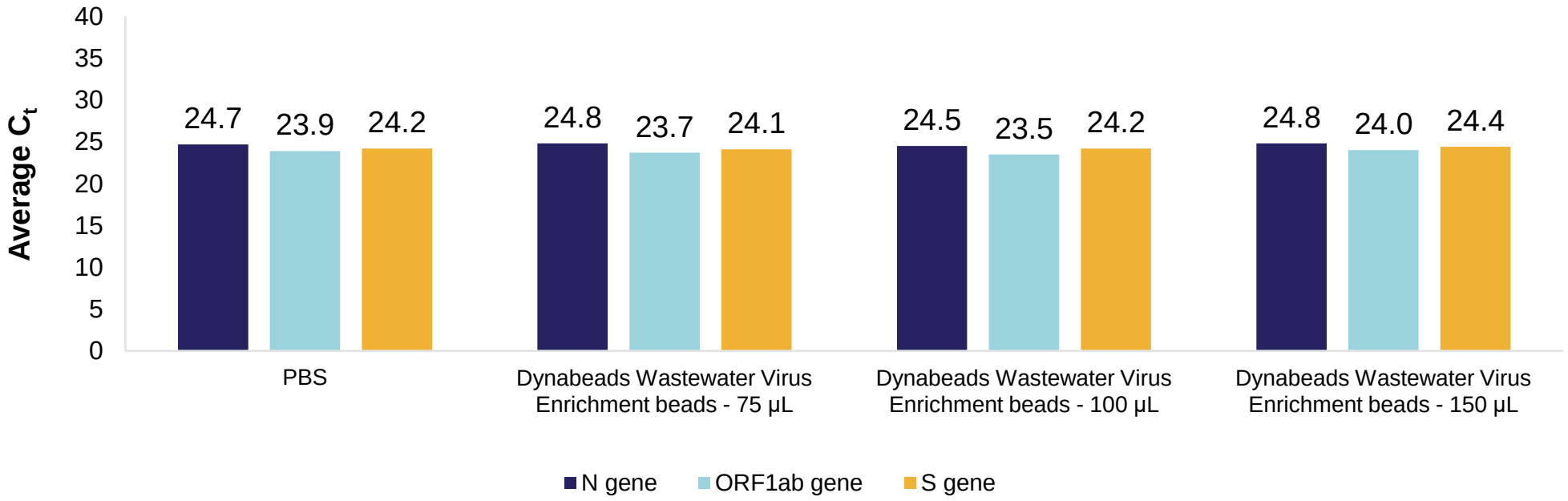
Addition of Proteinase K, binding buffer, and beads from MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment

Separation of beads using DynaMag magnet

Results for 10 mL wastewater samples

1 **SARS-CoV-2 nucleic acid extraction from 10 mL wastewater samples after automated pre-processing with magnetic beads**

- PBS control
- 4,000 copies in PCR

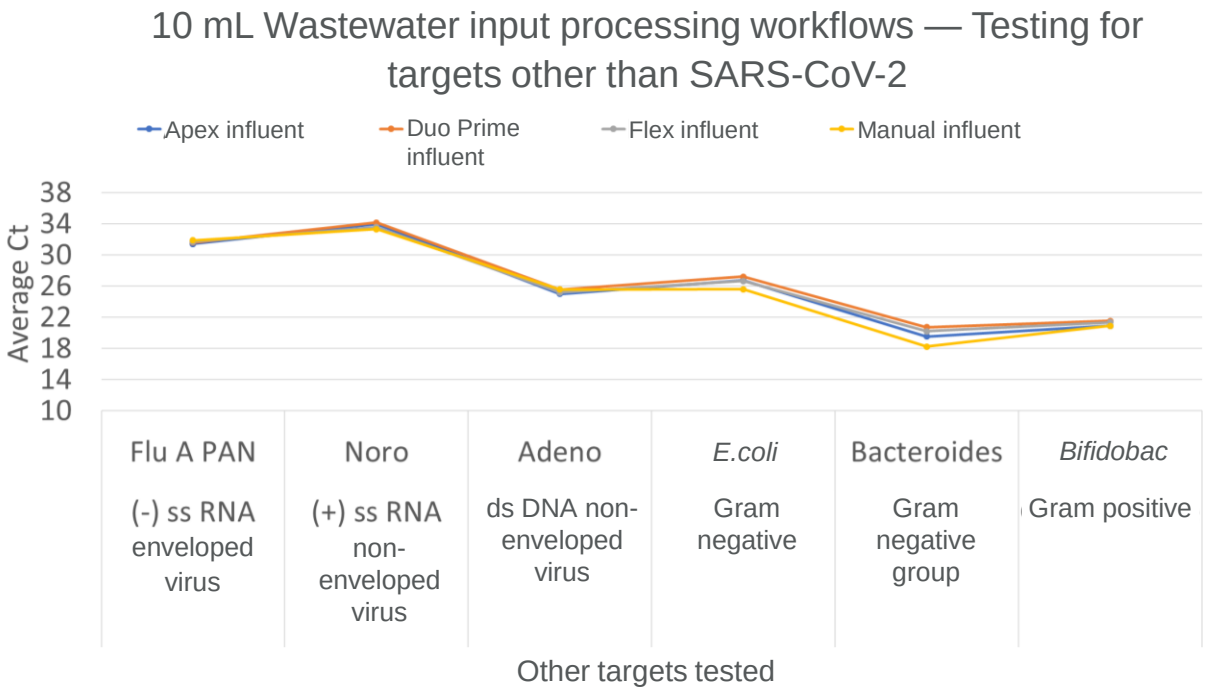


Viral load detected with spike-in of inactivated SARS-CoV-2 from 10 mL wastewater samples using Dynabeads Wastewater Virus Enrichment beads

10 mL Workflow testing on KingFisher Flex, Apex, Duo Prime instruments and manual process

10 mL Wastewater input with respiratory panel (RP) control spike-ins (ZeptoMetrix™)

1 **Applied Biosystems™ TaqMan® Assays for other viral and pathogen targets**



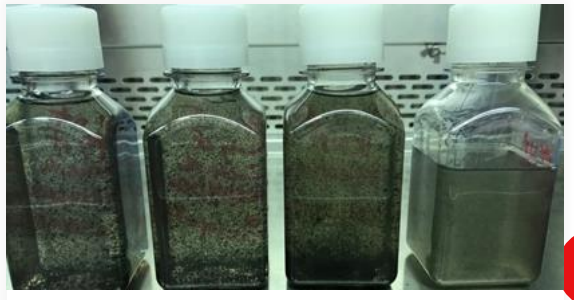
- 20% Elution in qPCR
- Applied Biosystems™ TaqMan® Fast Virus 1-Step Master Mix

Result:

Equivalent performance of all scripts and manual processing also for 10 mL wastewater input

Nucleic acid extraction

2 Protocol for 200 µL pre-concentrated* wastewater samples—direct method



Wastewater samples

Wastewater* samples taken after pre-processing for 5 min to a 96-deep-well plate

Extraction of nucleic acid with the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit

Downstream analysis

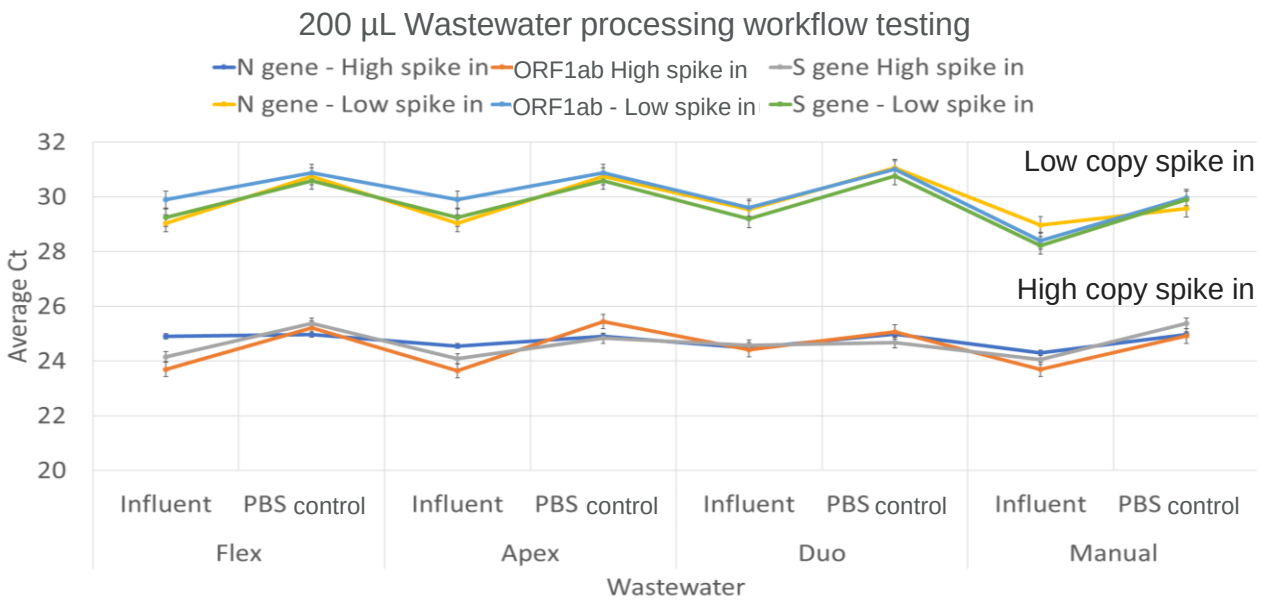
* Wastewater should be concentrated to a volume of 200 µL prior to starting this protocol, typically via ultracentrifugation.

Contrived & pre-processed wastewater samples @ 200ul per well + 200ul Lysis buffer

Workflow testing for 200 µL wastewater samples

Downstream analysis by real-time reverse transcription PCR (qRT-PCR)

2 **TaqMan assays for *N*, *ORF1ab*, and *S* genes of SARS-CoV-2:**



- PBS – Control
- 4,000 copies and 200 copies in PCR

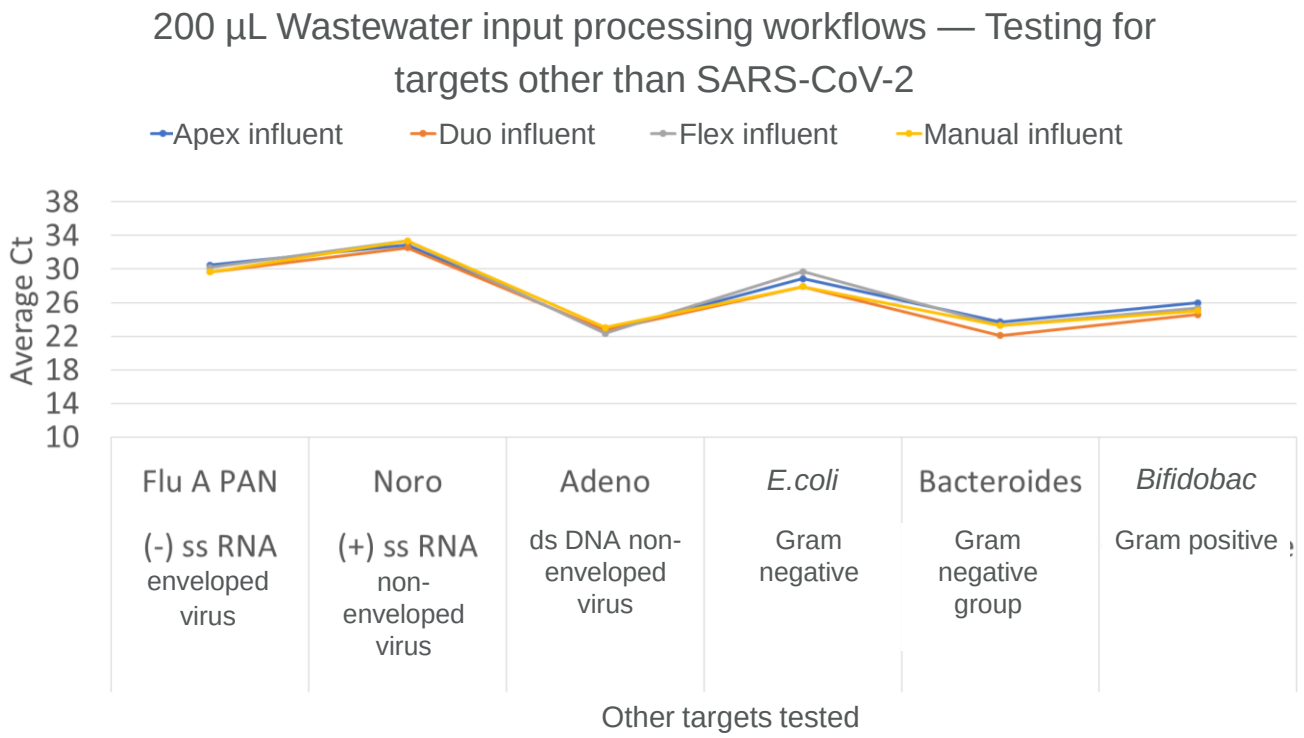
Result:

Equivalent performance of all scripts and manual processing also for 200 µL wastewater input for SARS-CoV-2 targets

Workflow testing on Flex, Apex, Duo Prime and manual processes

Other viral targets showed efficient recovery with the nucleic acid isolated from Zeptometrix RP contrived wastewater samples; gram positive and negative targets were also isolated efficiently

2 **TaqMan assays for other viral and bacterial targets**

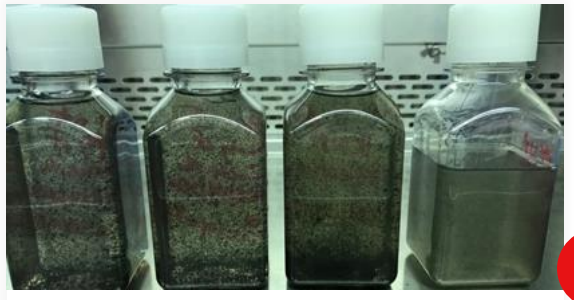


- 20% Elution in qPCR
- TaqMan Fast Virus 1-Step Master Mix

RP1 and RP2 spike-ins showed efficient recovery and bacterial targets (both gram positive and gram negative) also showed recovery without any spike-in

Nucleic acid extraction

3 Protocol for 1 mL wastewater samples and sludge



Wastewater samples

Wastewater* samples taken after pre-processing for 5 min to a 24-deep-well plate

Extraction of nucleic acid with the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit

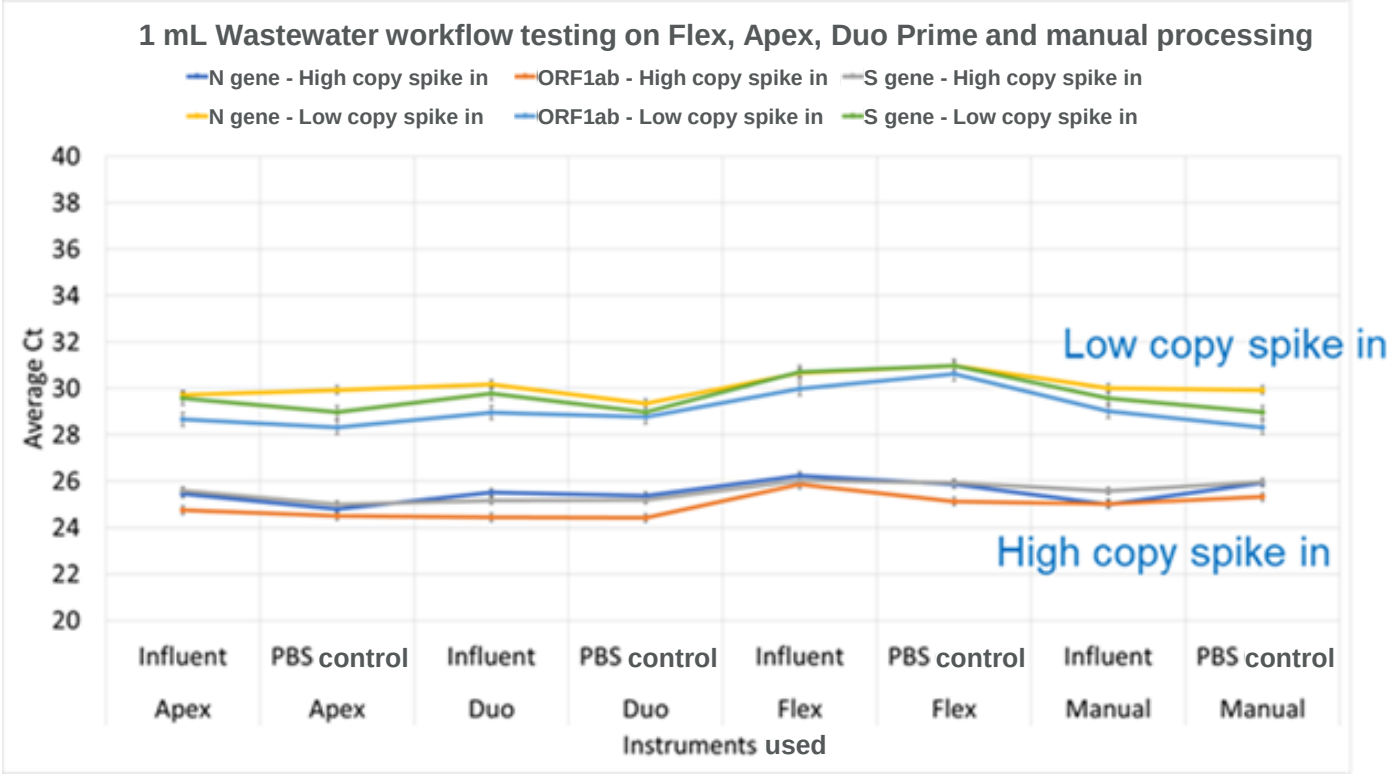
Downstream analysis

* Pre-processed wastewater samples with SARS-CoV-2 spike-in 1 mL per well + 1 mL lysis buffer

Results for direct method using 1 mL wastewater samples

3 TaqMan assays for *N*, *ORF1ab*, and *S* genes of SARS-CoV-2:

- PBS control
- 4,000 copies in PCR

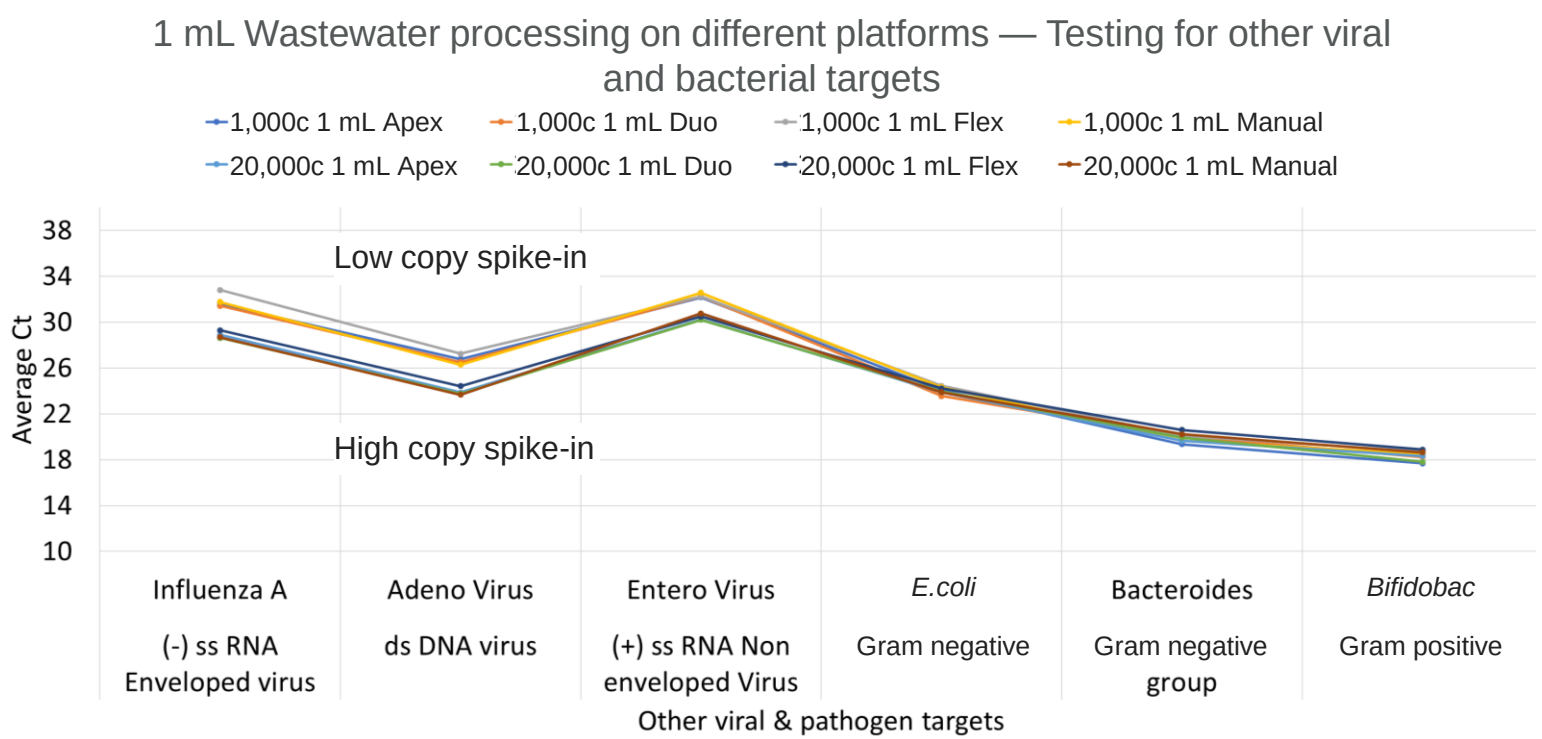


Viral load detected with spike-in of inactivated SARS-CoV-2;
1 mL wastewater samples have similar C_t and recovery data compared to the control sample

1 mL Workflow testing on KingFisher Flex, Apex, Duo Prime instruments and manual process

1 mL Wastewater input with RP control spike-ins (ZeptoMetrix)

3 TaqMan assays for other viral and bacterial targets

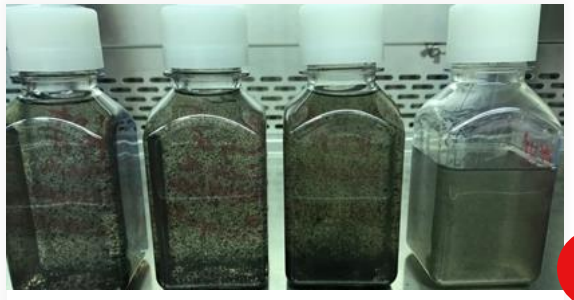


Result:

Equivalent performance of all scripts and manual processing also for 1 mL wastewater input for other viral and bacterial target detection

Nucleic acid extraction

4 50–500 mL samples using filtration methods for concentration



Wastewater samples

Wastewater* filter cake taken after pre-processing into a 24-deep-well plate

Extraction of nucleic acid with the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit

Downstream analysis

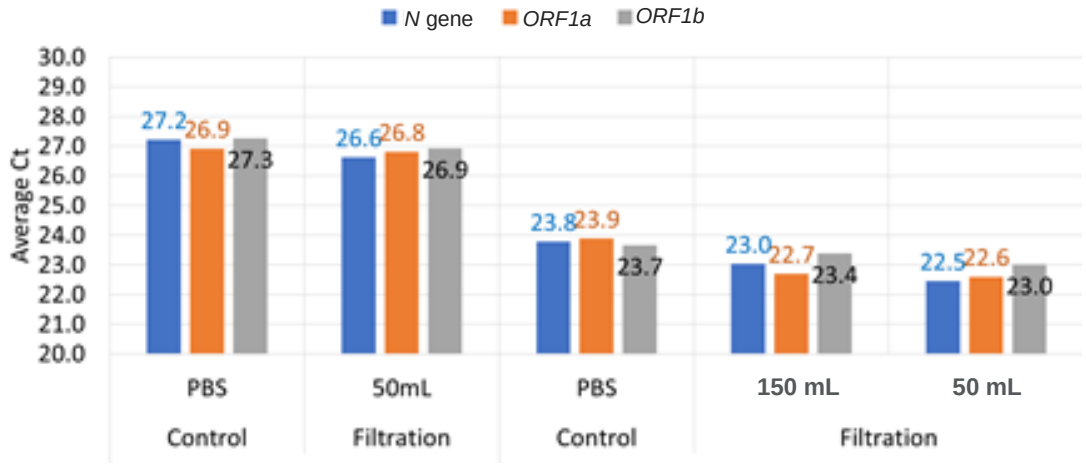
* 50–500 mL wastewater, filtered and pre-processed, with Lysis buffer and proteinase K used for isolation

Workflow testing for filtration method

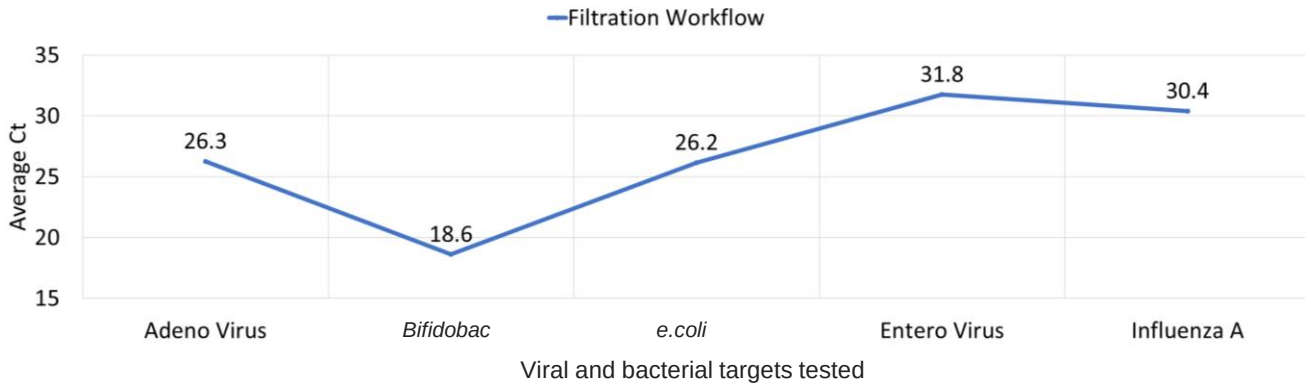
50–500 mL wastewater, filtered and pre-processed, and 2 mL lysis buffer used for isolation

4 **TaqMan assays for *N*, *ORF1ab*, and *S* genes of SARS-CoV-2 and other targets (viral and pathogen)**

Filtration for enrichment of SARS-CoV-2 for 50 mL wastewater



Filtration workflow for 50 mL wastewater processing for other viral and bacterial targets



First two columns in the graph are for Low copy spike in and Last three are for high copy spike in.

Result:
Filtration workflow works for >50 mL wastewater input

Nucleic acid extraction

5 200 mg sludge

Sludge samples

200 mg sludge taken after pre-processing for 5 min to a 24-deep-well plate

Pre-processed sludge with
SARS-CoV-2 spike-in
1 mL per well + 1 mL lysis buffer

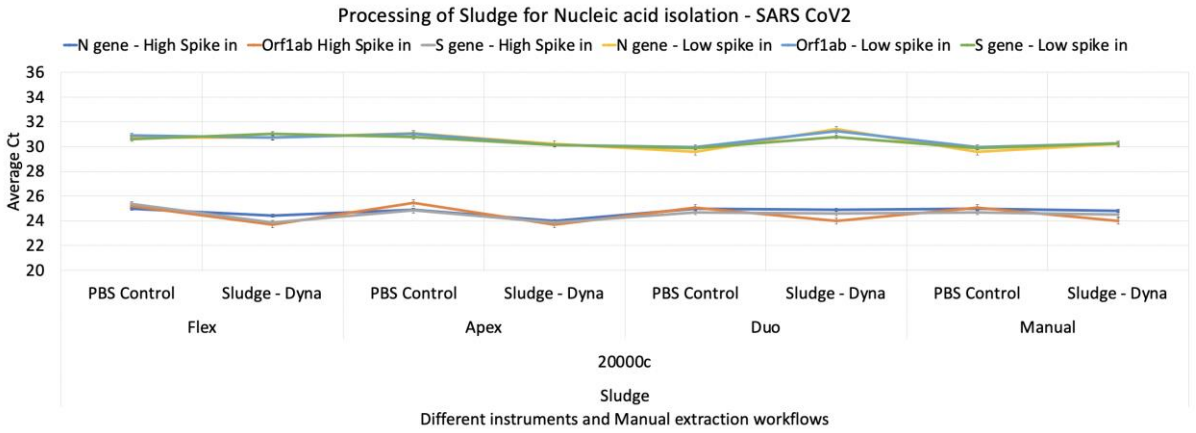
Extraction of nucleic acid with the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit

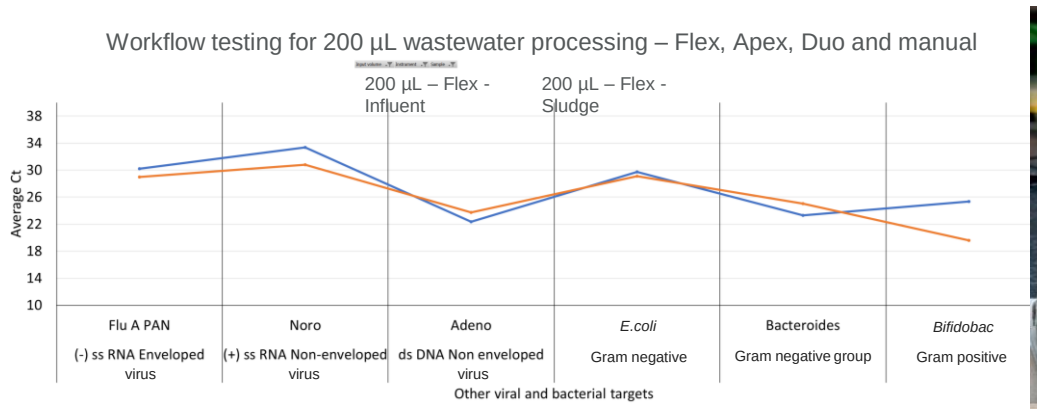
Downstream analysis

Workflow testing for sludge

200 mg sludge in 2 mL lysis buffer used for isolation

5 **TaqMan assays for *N*, *ORF1ab*, and *S* genes of SARS-CoV-2 and other targets (viral and pathogen)**





- PBS control
- 4,000 copies in PCR

Result:

200 mg sludge with 2 mL lysis buffer with pre-processed supernatant works well with the 1 mL workflow

Summary of sample processing at various input volumes

	Wastewater starting volume	Sample details	Kit reagents sufficient for	KingFisher scripts available	Protocol time	Purchase this kit	
1	10 mL	Concentration of 10 mL wastewater using Dynabeads followed by nucleic acid isolation	100 preps	Flex, Duo Prime, APEX	Less than 2 hours	MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment	A52610
2	200 µL	200 µL wastewater pre-concentrated using a preferred method, e.g., ultracentrifugation	100 preps	Flex, Duo Prime, APEX	45 minutes	MagMAX Wastewater Ultra Nucleic Acid Isolation Kit	A52606
3	1 mL	A. 1 mL non-concentrated wastewater B. Wastewater pre-concentrated to 1 mL volume using a preferred method, e.g., precipitation	20 preps	Flex, Duo Prime, APEX	45 minutes		
4	50 mL or more	Concentration of 10 mL wastewater using filtration followed by nucleic acid isolation	20 preps	Flex, Duo Prime, APEX	90 minutes		
5	Sludge	200 mg sludge	20 preps	Flex, Duo Prime, APEX	45 minutes		

How to order

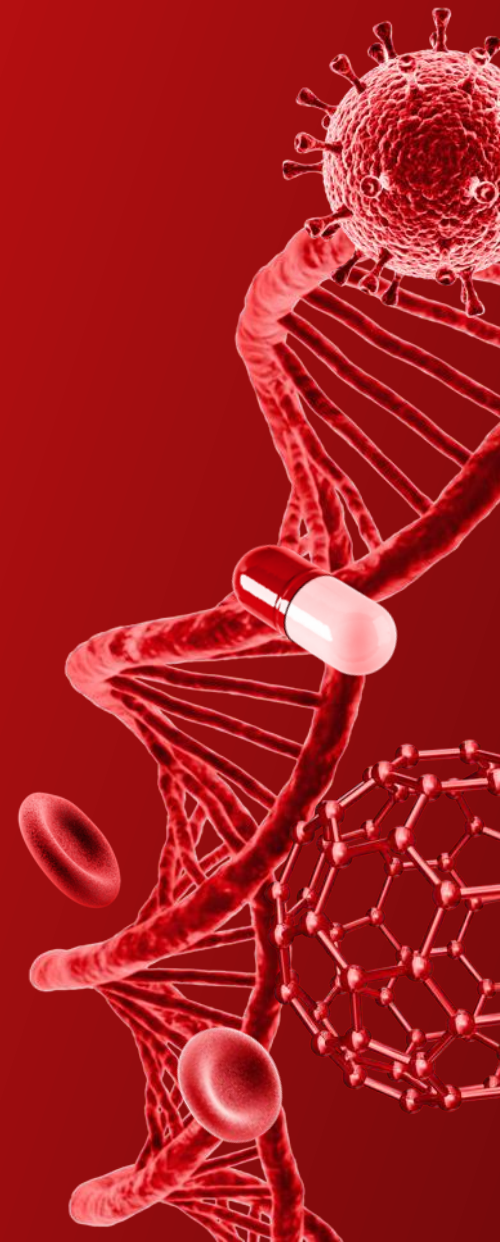
MagMAX Wastewater Ultra Nucleic Acid Isolation Kit

A52606

MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, with Virus Enrichment

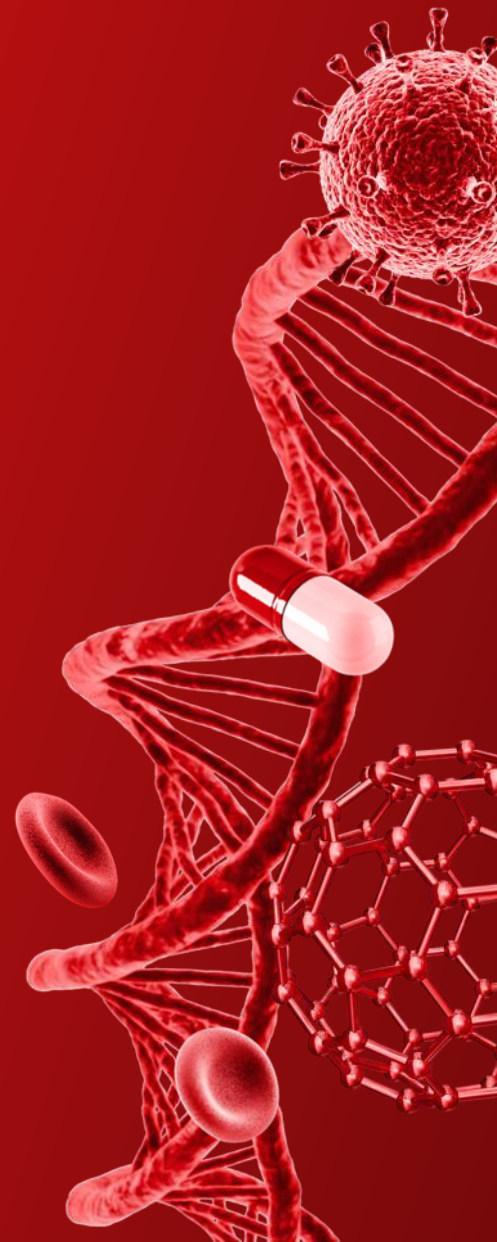
A52610

Thank you



Appendix

One slide per product mentioned under each workflow



Plastics needed for protocols

Protocol number	Wastewater starting volume	Preps per kit	Plate size used for virus enrichment	Plate size used for nucleic acid isolation	Number of KingFisher plates needed for full protocol (concentration + extraction)
1	10 mL	100	24-deep-well	96-deep-well	• Three 24-deep-well plates along with a tip comb plate for concentration • Six 96-deep-well plates along with a tip comb plate for isolation
2	200 µL	100	NA	96-deep-well	Six 96-deep-well plates along with a tip comb plate for isolation
3	1 mL	20	NA	24-deep-well	Four 24-deep-well plates along with a tip comb plate
4	≥50 mL	20	NA	24-deep-well	Four 24-deep-well plates along with a tip comb plate
5	Sludge	20	NA	24-deep-well	Four 24-deep-well plates along with a tip comb plate

Other methods for concentration during sample processing

The following methods are not as sensitive as using magnetic bead technology and are suboptimal for viral concentration

- Ultracentrifugation at 200,000 x *g* for 1 hour
  (Wurtzer et al. 2020)
- Precipitation with polyethylene glycol (PEG) 8000
  (Wu et al. 2020)
- Precipitation with ammonium sulfate (reported for cell culture only but researchers are testing for wastewater)

Downstream workflow Cat. Nos.

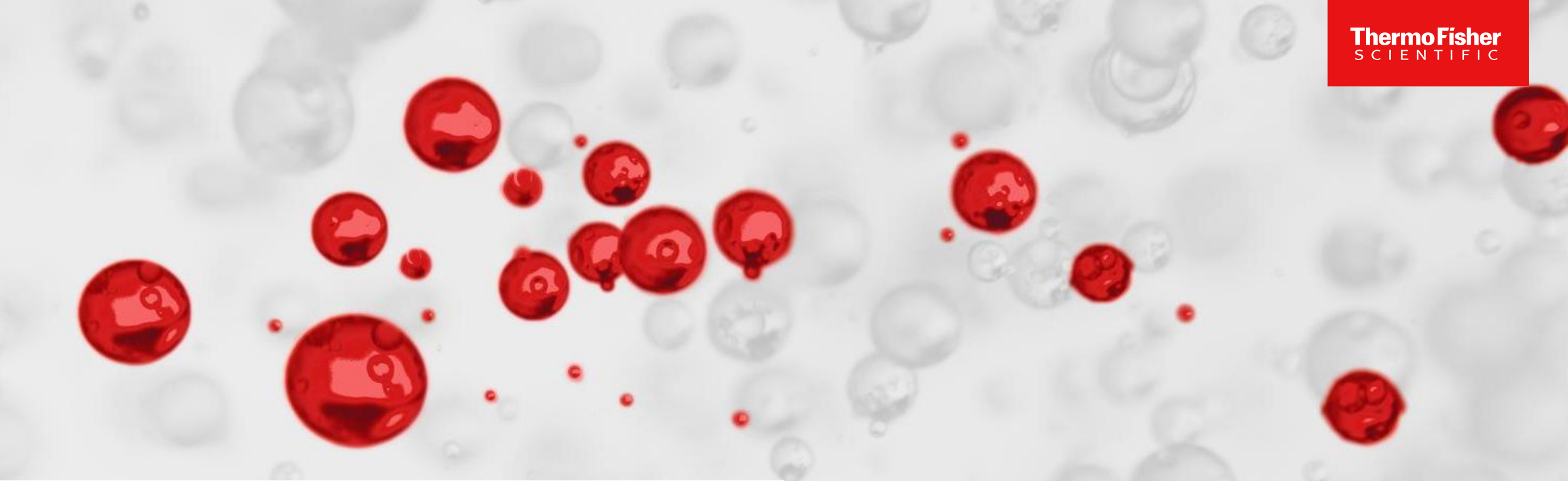
Cat. No.	Name	Size	Comment
A51121	TaqMan SARS-CoV-2 with RNase P Assay 2.0	1,000 rxn	Includes RNase P in assay tube
956129	TaqMan SARS-CoV-2 Plus Control	10 x 10 µL	*ORF1a*, *ORF1b*, and *N* gene RNA control
A49889	TaqMan SARS-CoV-2 RNA Control Dilution Buffer	10 x 250 µL	Dilution buffer for all RNA controls
A51327	TaqMan SARS-CoV-2 MS2 Assay 2.0	1,000 rxn	Includes MS2 phage control
956129	TaqMan SARS-CoV-2 Plus Control	10 x 10 µL	*ORF1a*, *ORF1b*, and *N* gene RNA controls
A49889	TaqMan SARS-CoV-2 RNA Control Dilution Buffer	10 x 250 µL	Dilution buffer for all RNA controls
A28523	TaqPath 1-Step qRT-PCR Master Mix (No ROX)	10 mL	Master mix for up to four different targets in a single multiplex reaction

Published literature and protocols for wastewater testing

- cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html
- who.int/news-room/commentaries/detail/status-of-environmental-surveillance-for-sars-cov-2-virus
- cen.acs.org/environment/water/Monitoring-COVID-19-sewage/98/i45
- sciencemag.org/news/2020/04/coronavirus-found-paris-sewage-points-early-warning-system
- nature.com/articles/s41587-020-0620-2
- nature.com/articles/s41893-020-00605-2
- nature.com/articles/d41586-020-00973-x
- nature.com/articles/s41545-020-0079-1
- nature.com/articles/s41587-020-0684-z
- sciencedirect.com/science/article/abs/pii/S0043135420310952
- sciencedirect.com/science/article/pii/S2666379120301245

References

- Wurtzer S, Marechal V, Mouchel J et al. (2020) Evaluation of lockdown impact on SARS-CoV-2 dynamics through viral genome quantification in Paris wastewaters. medRxiv doi:10.1101/2020.04.12.20062679.
- Wu F, Xiao A, Zhang J et al. (2020) SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. medRxiv doi:10.1101/2020.04.05.20051540.
- Ahmed W, Angel N, Edson J et al. (2020) First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. *Science of the Total Environment* 728:138764.
- Nemudryi A, Nemudraia A, Wiegand T et al. (2020) Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater. *Cell Reports Medicine* 1:100098.



Ultracentrifugation

Concentration of SARS-CoV-2 RNA in wastewater samples for COVID-19 surveillance

Ultracentrifugation method:

Thermo Scientific™ Sorvall™ WX+ Ultracentrifuge and SureSpin™ 630 Swinging Bucket Rotor

Transfer 20 mL of wastewater samples into Thermo Scientific™ 36 mL PET Thin-walled Ultracentrifuge Tubes

Add 12 mL sucrose cushion underneath aliquoted samples

Centrifuge at 150,000 x *g* for 45 min at 4°C

Discard supernatant, resuspend pellet in 200 µL 1X PBS

Perform qPCR for detection and quantification

Sucrose cushion: 50% sucrose in TNE buffer (20 mM Tris- HCL (pH 7.0), 100 mM NaCl, 2 mM EDTA)

Sorvall WX+ Ultracentrifuge

SureSpin 630 Swinging Bucket Rotor (replaced by SureSpin™ 632 rotor)

Methodology retrieved from Wilder et al. *Water Research* X. 2021.

Concentration of SARS-CoV-2 RNA in wastewater samples for COVID-19 surveillance

Superspeed centrifugation method:

Thermo Scientific™ Sorvall™ LYNX™ 6000 Superspeed Centrifuge and Fiberlite™ F12-6 x 250 LEX rotor

Transfer 200 mL of samples into 500 mL Fiberlite PPCO Thermo Scientific™ Nalgene™ Oak Ridge bottles

Centrifuge at 7,140 x *g* for 15 min at 4°C

Filter supernatant through 0.22 µm filter and precipitate with PEG and NaCl overnight at 4°C in 500 mL Nalgene Oak Ridge bottles

Centrifuge precipitated filtrates at 16,900 x *g* for 30 min at 4°C

Resuspend pellet in 2 mL 1X PBS

Perform qPCR for detection and quantification

PEG (8% w/v, 16 g)

NaCl (0.5 M, 5.844 g)

Sorvall LYNX 6000 Superspeed Centrifuge

Fiberlite F12-6 x 250 LEX rotor

Methodology retrieved from Stadler et al. medRxiv 2020, accessed on 07.05.2021 https://www.medrxiv.org/content/10.1101/2020.11.04.20226191v1

Concentration of SARS-CoV-2 RNA in wastewater samples for COVID-19 surveillance

Centrifugal ultrafiltration method:

Thermo Scientific™ general purpose centrifuges and swing-out bucket rotors

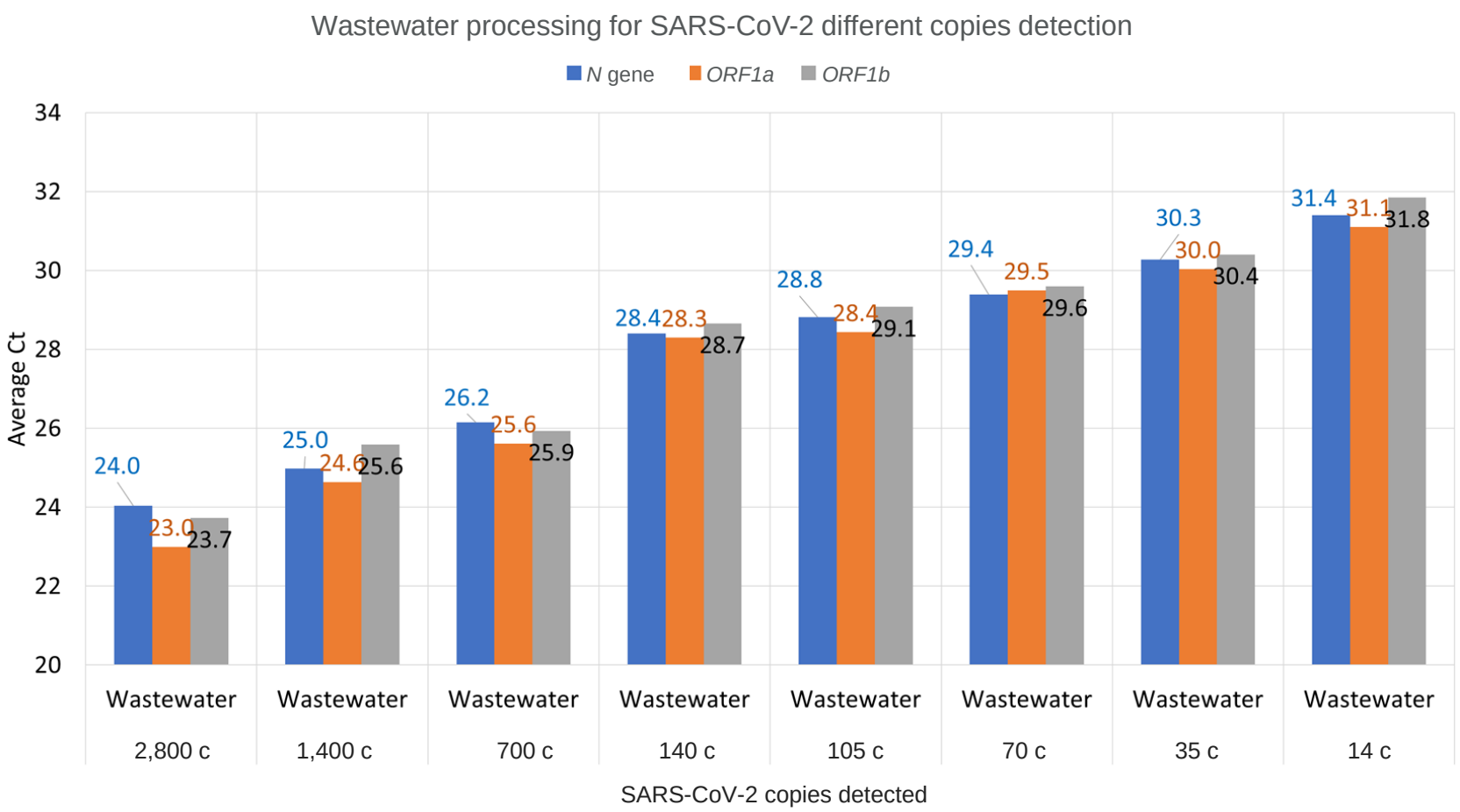
Transfer samples into 50 or 250 mL conical tubes → Centrifuge at 4,000–5,000 x *g* for 30 min to remove larger particles → Filter 100–200 mL of supernatant through a centrifugal ultrafilter (100 kDa) by centrifuging at 1,500 x *g* for 15 min (3,500 x *g* with 10 kDA filters) → Extract RNA → Perform qPCR for detection and quantification

Rotor	TX-1000	TX-750	BIOShield™ 1000A	TX-400
Ultrafilter capacity	8	4	4	4

Methodology retrieved from Medema et al., *Environ Sci Technol Lett*., 2020 and Ahmed et al., *Sci Total Environ*., 2020.

Detection limit for SARS-CoV-2 with wastewater workflow

200 µL wastewater input experiment



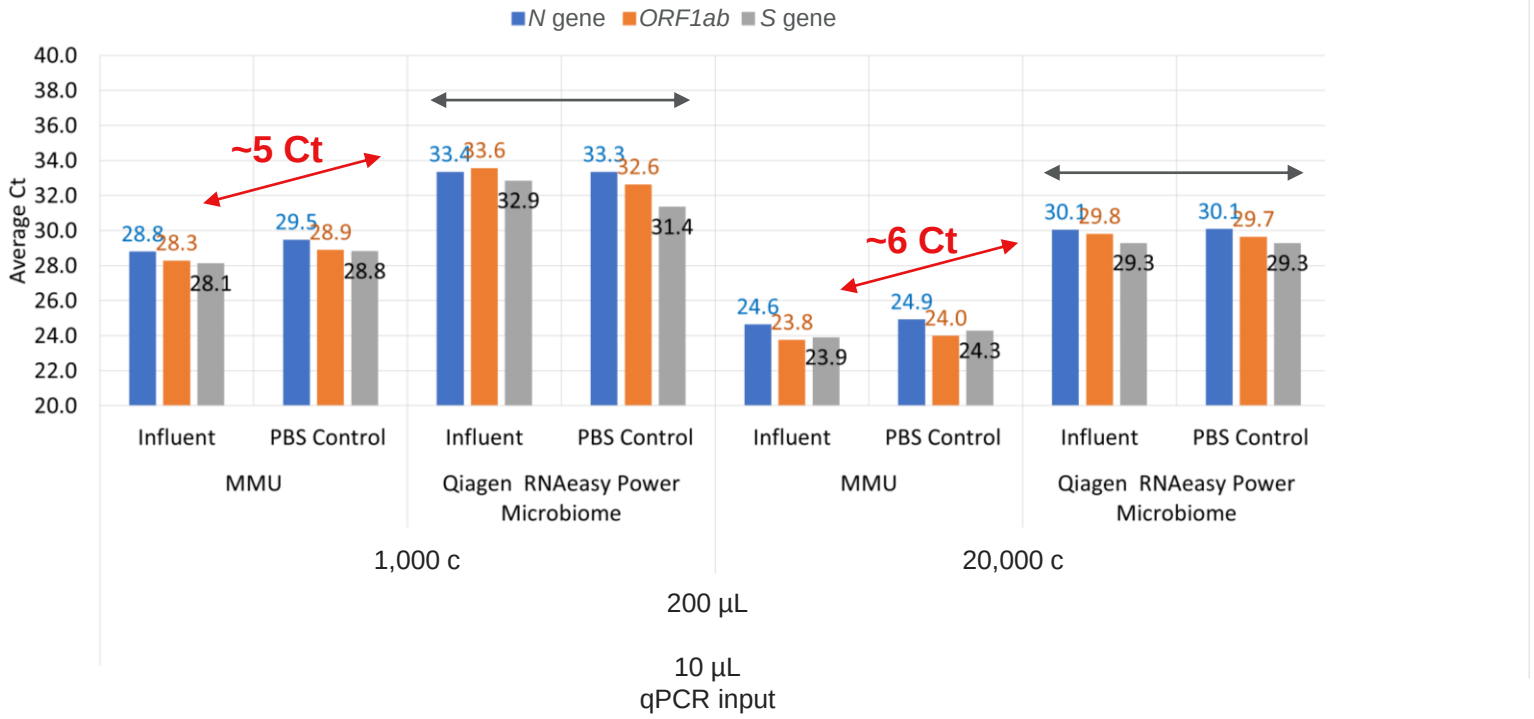
Wastewater kit was able to isolate as low as 14 copies of SARS-CoV-2 from contrived 200 µL wastewater samples

Comparison with QIAGEN™ RNeasy™ PowerMicrobiome™ Kit

200 µL wastewater input processed with both MagMAX Wastewater Ultra and QIAGEN kits

TaqMan assays for *N*, *ORF1ab*, and *S* genes of SARS CoV2

Comparison of MagMAX Wastewater Kit vs. QIAGEN RNeasy PowerMicrobiome Kit for 200 µL wastewater



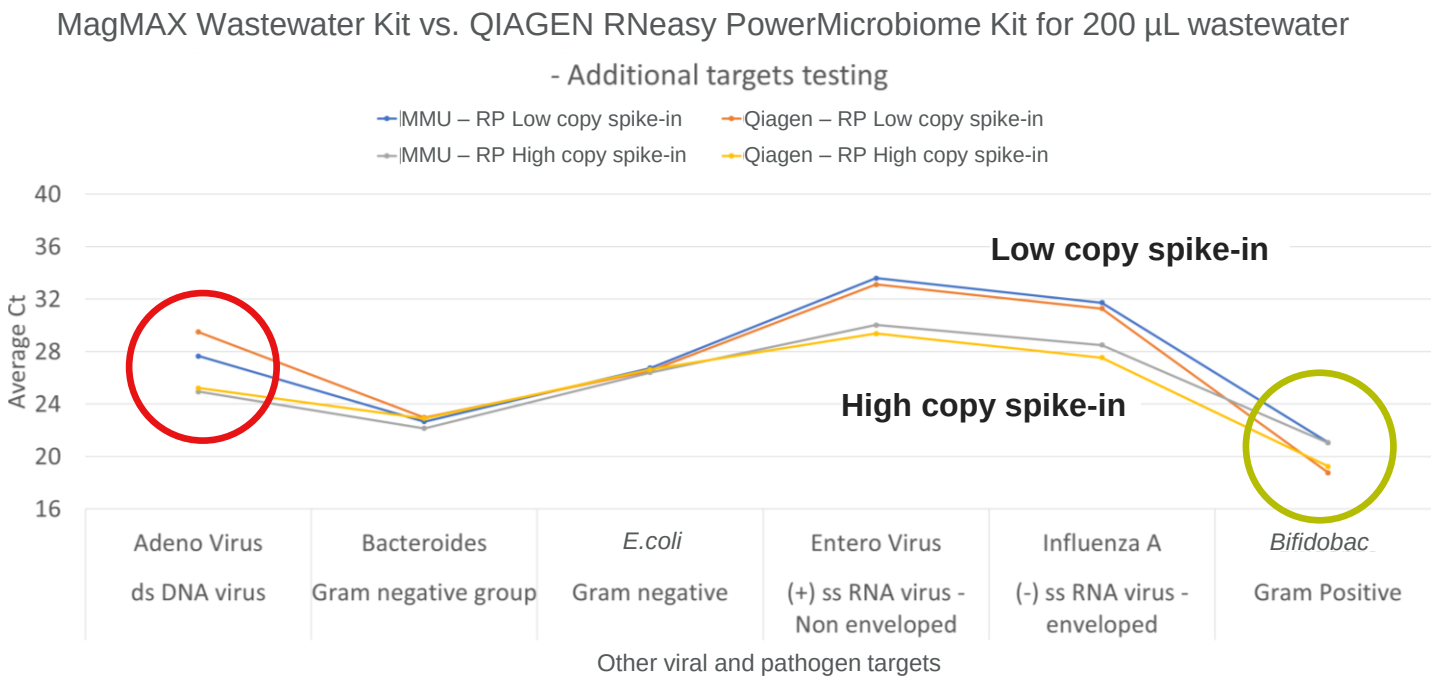
Result:

MagMAX Wastewater Ultra Kit demonstrated superior performance than the QIAGEN kit for high and low copies of SARS-CoV-2 nucleic acid isolation from 200 µL wastewater

Comparison with QIAGEN RNeasy PowerMicrobiome Kit for other viral and bacterial targets

200 µL wastewater input with RP control spike-ins (ZeptoMetrix)

TaqMan assays for *N*, *ORF1ab*, and *S* genes of SARS-CoV-2



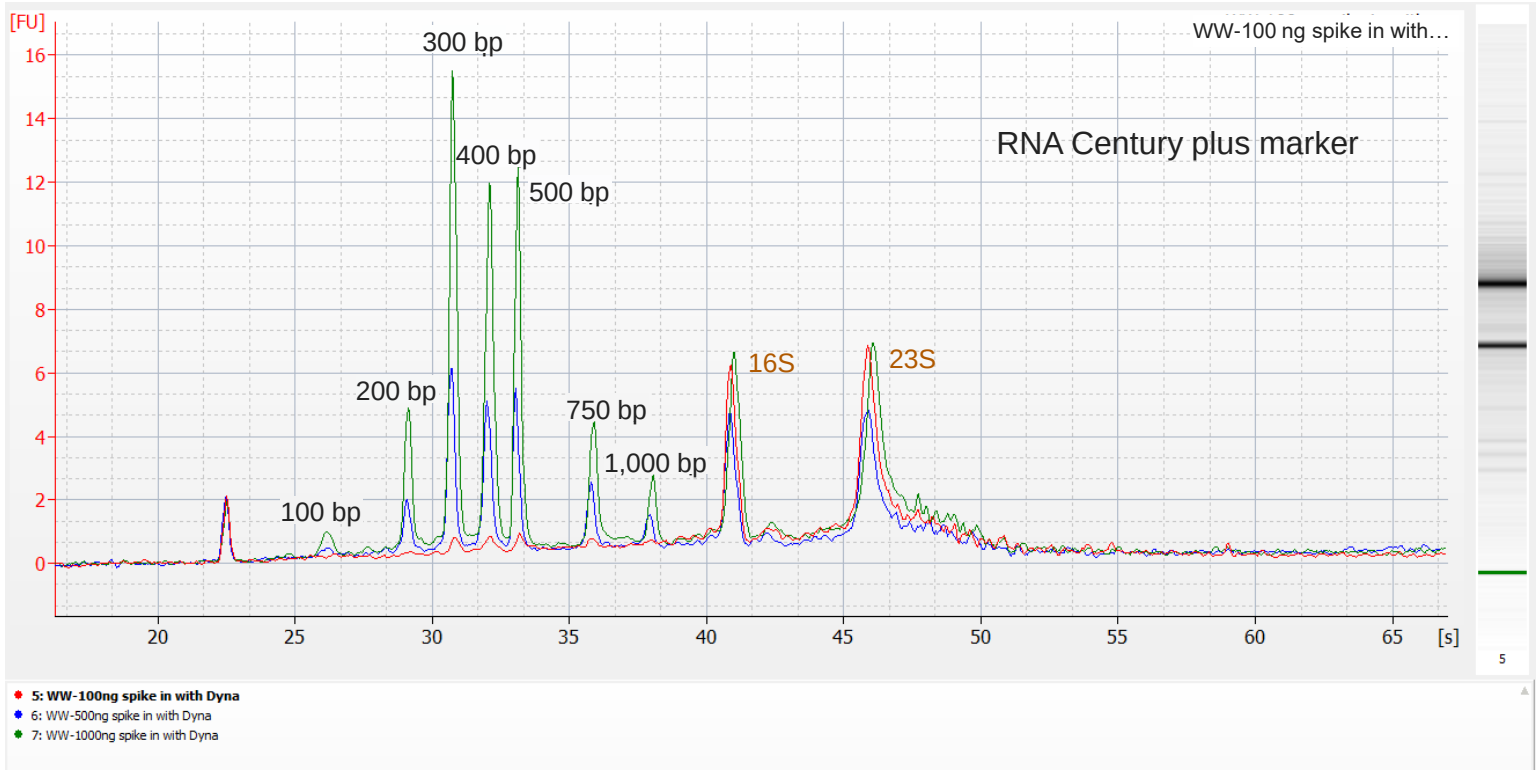
Result for Adenovirus—MagMAX Wastewater Ultra kit is better

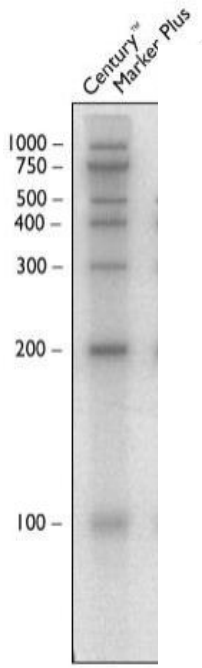
For Gram positive bacteria—QIAGEN kit is better because of bead-beating; for other targets tested, both kits are giving equivalent performance

Do Dynabeads bind fragmented RNA as well?

1 mL wastewater with 100–1000 ng Applied Biosystems™ Ambion™ RNA Century™-Plus Markers enriched with 20 µL Dynabeads

BioAnalyzer trace on RNA pico chip





Result:

Dynabeads are binding fragmented RNA as well as virus.

Wastewater sample extractions show good quality of RNA with RNA Integrity Number (RIN) value of ~7.0